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Original Article

Increased chemokine ligand 26 expression and its involvement in epithelial-mesenchymal transition in the endometrium with adenomyosis

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ABSTRACT

Objective: Adenomyosis is a gynecologic disorder characterized by symptoms of dysmenorrhea, abnormal uterine bleeding, and infertility. This study aimed to analyze the expression profiles of key inflammatory cytokines in the endometrium with adenomyosis and their involvement in epithelial-mesenchymal transition (EMT).
Study Design: Endometrial tissues collected from premenopausal women with (n = 3) or without (n = 3) adenomyosis during the secretory phase were subjected to DNA array analysis to examine inflammatory cytokines. The gene and protein expression levels were re-evaluated by reverse transcription-polymerase chain reaction (n = 19) and immunohistochemistry (n = 56). Immunohistochemical analysis using the Histo-scores of chemokine ligand 26 (CCL26) and EMT-related factors was performed with uterine tissues resected for adenomyosis (n = 37), including those from patients treated with gonadotropin-releasing hormone agonist (GnRHa). An invasion assay was also performed using endometrial epithelial cells.
Results: DNA array results showed that *CCL26, IL-1B,* and *CCL3* were upregulated. *CCL26* mRNA expression was markedly higher in the endometrium with adenomyosis than in that without adenomyosis. Immunohistochemical analysis revealed that CCL26 expression was elevated in the epithelial cells of the basal layer of the endometrium with adenomyosis than in that without adenomyosis regardless of GnRHa treatment. In the basal layer of the endometrium with adenomyosis, CCL26 expression was positively correlated with neural-cadherin and ZEB1 expression; additionally, the cases with intrinsic-type adenomyosis had high expression levels of CCL26 and ZEB1. Exogenous CCL26 promoted the invasive activity of endometrial epithelial cells.
Conclusions: CCL26, an inflammatory mediator, may be involved in the pathogenesis of adenomyosis by inducing EMT in the basal layer of the endometrium.

Introduction

Adenomyosis is characterized by the benign invasion of ectopic endometrium into the myometrium. The most widely accepted hypothesis for the pathogenic mechanism of adenomyosis is that it develops as a down-growth and invagination of the basalis endometrium into the myometrium [1,2]. Epithelial-mesenchymal transition (EMT) is a biological process involved in embryological development, wound healing, tissue regeneration, and cancer progression [3]. In EMT, epithelial cells obtain the characteristics of mesenchymal cells by acquiring invasive properties, and this mechanism is presumably involved in the etiology of adenomyosis.

Although the recent advancements in non-invasive diagnostic tools, i.e., ultrasonography (US) and magnetic resonance imaging (MRI), have been practical, adenomyosis is often diagnosed by histology after hysterectomy. Additionally, there is currently no method for evaluating the endometrial tissues with adenomyosis. As such, useful markers of adenomyosis are needed to assess the disease's state and determine the treatment plan for outpatients.

We focused on the crucial factors in the basal layer of the endometrium with adenomyosis as the endometrial and myometrial interface. Uterine auto-traumatization and the initiation of the mechanism of tissue injury and repair (TIAR) were considered the primary events in the disease process [4]. In the disease's early developmental stage, adenomyosis lesions may result from the activation of the TIAR mechanism and the subsequent inflammation and infiltrative growth beyond the

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interface. To explore the inflammatory factors involved in the pathogenesis of adenomyosis, we examined the gene expression profiles of inflammatory cytokines in the endometrial tissues with adenomyosis and their correlation with EMT-related factors. Notably, chemokine ligand 26 (CCL26/eotaxin-3) expression was significantly enhanced in the endometrial tissues with adenomyosis. CCL26 is known to be an inflammatory cytokine involved in eosinophil migration as it acts on the C—C motif chemokine receptor 3 (CCR3). It has been reported that CCL26 promotes EMT signaling pathway in colon cancer cells. CCL26 siRNAs suppressed the expression of tissue inhibitor matrix metalloproteinase 1 (TIMP1), nicotinamide N-methyltransferase (NNMT), and fibromodulin (FMOD), while CCL26 overexpression increased the expression of all. [5]

We investigated the effects of CCL26 on EMT-like processes in the epithelial cells of the basal layer of the endometrium with adenomyosis. We also sought to analyze the expression of CCL26 in the different types of adenomyosis lesions with or without gonadotropin-releasing hormone agonist (GnRHa) pretreatment or the coexistence of ovarian endometrioma (OE).

Material and methods

Patients and tissue samples

This study was approved by the institutional review board of Tottori University Faculty of Medicine (No. 19A043), and all patients provided written informed consent. Endometrial tissues were obtained from 19 women with adenomyosis ($n = 12$) or without adenomyosis ($n = 7$) who visited our hospital as outpatients in 2019. For gene expression analysis, we collected specimens of endometrial tissues using an aspiration pipette (MedGyn Pipette IV; MedGyn, Addison, IL, USA).

Specimens for immunohistochemical analysis were collected from patients who underwent a hysterectomy between 2010 and 2019; we obtained 37 specimens from patients with adenomyosis and 19 specimens from patients without adenomyosis. According to MRI findings, we categorized adenomyosis patients into two subtypes, i.e., the (a) intrinsic type, if they were seen at the inner myometrium affecting the junctional zone, and normal myometrium is recognizable on the outer side of the foci, or (b) extrinsic type, if they were seen focally at the outer myometrium without affecting the junctional zone, where normal myometrium is recognizable on the inner side of the foci [6,7]. Extrinsic type includes invasive endometriosis lesions from outside the uterus, i.e., deep endometriosis (DE) [8]. We diagnosed adenomyosis, OE, and uterine fibroids from the findings of US or MRI, then confirmed pathologically after hysterectomy. In the patients with adenomyosis, we evaluated the pain related to menstruation before hysterectomy using a numeric rating scale (NRS), a method for self-rating pain on a scale of 0–10 points.

DNA array

Endometrial tissues in the secretory phase with adenomyosis ($n = 3$) and without adenomyosis ($n = 3$) were analyzed using a DNA array to examine the inflammatory cytokine profile (RT [2] Profiler PCR Array, Human Inflammatory Cytokines & Receptors; QIAGEN, Tokyo, Japan). This array can be used to assess the expression profiles of 84 key genes that mediate the inflammatory response. Total RNA (500 ng) was reverse-transcribed into complementary DNA, and the analysis was performed according to the manufacturer's instructions.

Reverse transcription (RT)-PCR

Endometrial tissues with adenomyosis ($n = 12$; proliferative, $n = 4$, and secretory phase, $n = 8$) or without adenomyosis ($n = 7$; proliferative, $n = 3$, and secretory phase, $n = 4$) were used. Total RNA (200 ng) was reverse-transcribed into complementary DNA using the RT [2] First Strand Kit (QIAGEN). TaqMan probes (Thermo Fisher Scientific, Tokyo, Japan) for *CCL26*, chemokine ligand 3 *(CCL3)*, interleukin-1β (*IL-1B*), complement C5 (*C5*), and chemokine ligand 9 (*CXCL9*) were used. The mRNA levels were quantified using ViiA 7 real-time PCR system (Thermo Fisher Scientific). Absolute values for each probe were normalized to those for glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), and the relative mRNA levels are shown as the ratio to values of the control.

Immunohistochemical staining

We collected uterine specimens from 37 adenomyosis patients who had undergone a hysterectomy. Among these patients, 18 patients were treated with GnRHa (Group B), and 19 patients were not treated with GnRHa (Group A). In group A ($n = 19$), 16 cases of dysmenorrhea, and 4 cases of menorrhagia. One patient complained of both dysmenorrhea and menorrhagia. In group B ($n = 18$), 12 cases of dysmenorrhea, and 13 cases of menorrhagia. Seven patients complained of both dysmenorrhea and menorrhagia. Uterine specimens from patients diagnosed with an early stage of uterine cervical cancer (FIGO stage IA1) without adenomyosis were set in the control group (Group C).

All tissue sections were fixed with 10% formalin and embedded in paraffin. The procedure and primary antibodies (E-cadherin, N-cadherin, CCL26/eotaxin-3, CCR3, Snail, ZEB1: zinc-finger-enhancer binding protein 1, Twist) were shown in Table S1 and S2. We assessed the results by the semi-quantitative Histo-score (H-score). Staining intensity (0, +1, +2, or +3) was determined for each cell in a fixed field, then the percentage of cells at each staining intensity level was calculated. H-score was assigned using the following formula: [1 × (% cells 1+) + 2 × (% cells 2+) + 3 × (% cells 3+)]. We calculated the score in 10 random locations on each slide. For the evaluation of the expression of multiple factors, we used serial sections of the same paraffin-embedded specimen. We distinguished the basal and functional layers of the endometrial specimens according to the classification of Padykula et al. [9].

Invasion assay

To evaluate the effect of CCL26 on the invasion of endometrial cells, we performed an invasion assay using the Matrigel Invasion Chambers (Corning, NY, USA). Since it is difficult to continuously culture endometrial epithelial cells, we used Ishikawa cells, a well-differentiated endometrial adenocarcinoma cell line [10,11]. In the upper insert, 1.0×10^6 cells were seeded with serum-free Dulbecco's Modified Eagle Medium, and cultured with human recombinant CCL26 (1 μg/mL; R&D, MN, USA) for 24 h at 37C in a 5% CO_2 incubator ($n = 3$). Membranes were stained with Diff-Quik (Sysmex, Kobe, Japan), and the number of cells appearing on the undersurface of Matrigel was counted in three fields. The rate of invasion (%) was calculated as: [the number of cells that invaded the Matrigel/those that migrated to the control insert × 100].

Statistical analysis

The mean with the standard error (SE) and the variables using a two-tailed paired student's *t*-test was expressed. Nonparametric Mann-Whitney *U* test showing the median [25th, 75th percentile] or box-and-whisker plots were used. Lines inside of the boxes indicate the median values and the upper or lower limits of the boxes and whiskers indicate the interquartile and total ranges. All statistical analyses were carried out using JMP (SAS Institute, Cary, NC, USA). P values less than 0.05 were considered to indicate statistical significance.

Results

Gene expression of CCL26 and its receptors in endometrial tissues

In the DNA array, including those of inflammatory cytokines and receptors, the *CCL26, CCL3,* and *IL-1B* genes were upregulated, and the *C5* and *CXCL9* genes were downregulated in the endometrial tissues in the secretory phase with adenomyosis (Table 1). For these 5 genes, we performed RT-PCR to evaluate their levels in other specimens (Fig. 1A). Of those, only *CCL26* expression was markedly elevated in the endometrium with adenomyosis when compared to that without adenomyosis. In both the proliferative and secretory phases, the expression of *CCL26* was higher in the endometrium with adenomyosis than in that without adenomyosis, whereas the expression of *CCR3* (the main receptor for CCL26) was similar (Fig. 1B).

Immunoexpression of CCL26, CCR3, and cadherins

Characteristics of patients included are shown in Table 2. Seven (77.8%) of the 9 patients with extrinsic type and 5 (17.9%) of the 28 patients with intrinsic type in Groups A and B had coexisting OE. In these 12 patients with OE, the revised ASRM scores were similar regardless of GnRHa treatment (Group A: 67.1 ± 27.0; Group B: 71.2 ± 36.5) (data not shown).

NRS before hysterectomy, among patients with adenomyosis, the expression of CCL26 was significantly higher in the group with NRS of 8 points or more than that of less than 8 points (Fig. 2).

In the basal layer of the endometrium, the expression levels of CCL26 and neural (N)-cadherin were higher in the endometrium with adenomyosis and in adenomyotic lesions than in the endometrium without adenomyosis. In contrast, the epithelial (E)-cadherin expression level was elevated in the endometrium without adenomyosis when compared to the other two groups. The expression level of CCR3 was similar among the three groups (Fig. 3A and 3B).

When all adenomyosis patients were evaluated as a group (Groups A and B combined), a positive correlation between CCL26 expression and N-cadherin expression was observed, although no such correlation was found between CCL26 and E-cadherin (Fig. 3C). The expression levels of CCL26, N-cadherin, E-cadherin, and CCR3 were similar regardless of GnRHa treatment (Fig. 3D). Coexisting OE or fibroids did not influence the expression of CCL26, N-cadherin, or E-cadherin (Fig. 3E).

Table 1
DNA array for inflammatory cytokines in endometrium with or without adenomyosis.

Upregulated genes			Downregulated genes		
Gene symbol	Fold regulation	Evaluation	Gene symbol	Fold regulation	Evaluation
CCL26	2.26	A	*C5*	−2.64	A
CCL3	2.16	A	*CXCL9*	−1.71	A
IL1B	1.77	A	*CCR4*	−2.43	C
CCL8	2.52	B	*IL33*	−2.41	C
CCL4	2.32	C	*CCL22*	−2.10	B
IL17A	2.08	B	*CCL20*	−1.90	B
IL15	1.70	C	*CXCL13*	−1.81	B
IL1RN	1.70	C	*CXCL5*	−1.81	C
SPP1	1.70	C	*CXCL11*	−1.79	B
IL13	1.67	C	*CXCL8*	−1.68	C
CCL2	1.66	C	*CCL24*	−1.67	B
CX3CR1	1.64	B	*IL21*	−1.55	B

Note: "Fold regulation" indicates the relative mRNA expression level in the endometrial tissues with adenomyosis in comparison to those without adenomyosis. "A" indicates a significant difference in expression between the two groups ($C_t < 30$ and $C_t > 30$).
"B" indicates a low expression level in both groups ($C_t > 30$). "C" indicates no difference between the two groups ($C_t < 30$).
Abbreviations: Ct, cycle threshold.

Of the 37 patients in the adenomyosis group, 10 had DE, and 9 had ovarian endometrioma. Regardless of whether DE exists, there was no difference in the expression of CCL26, E-cadherin, and N-cadherin (data not shown).

Involvement of CCL26 and EMT-related transcription factors

Immunohistochemical images for EMT-related transcription factors, CCL26, ZEB1, and Snail and Twist, in the basal layer of the endometrium are shown (Fig. 3A). H-scores of ZEB1, as well as CCL26, were higher in the endometrium with adenomyosis than in that without adenomyosis (Fig. 3B), although Snail and Twist did not differ between them (Fig. 3A). A positive correlation was observed between the CCL26 and ZEB1 expression levels (Fig. 3C). According to the types of adenomyosis, representative immunohistochemical images for CCL26 and ZEB1 are shown (Fig. 3D). H-scores of CCL26 and ZEB1 were significantly higher in the endometrium of the intrinsic type than in the endometrium of the extrinsic type (Fig. 3E).

Using MRI findings, we diagnosed the phenotypes of adenomyosis as focal ($n = 23$) and diffuse ($n = 14$). A positive correlation of CCL26 and ZEB1 expression was observed within focal or diffuse type, although there was no significant difference in CCL26 or ZEB1 expression between the two types (data not shown).

Effect of CCL26 on endometrial cell invasion

RT-PCR analysis verified that Ishikawa cells expressed CCR3 mRNA, whereas the cultured endometrial stromal cells did not (data not shown). Exposure to recombinant CCL26 significantly promoted the invasive activity of Ishikawa cells (Fig. 4).

Fig. 5

Discussion

Enhanced CCL26 expression in the basal layer of the endometrium with adenomyosis, especially in the intrinsic type, was positively correlated with the EMT process, suggesting that CCL26 is a potential biomarker for the practical management of adenomyosis. CCL26 and EMT mechanism may affect the events involving TIAR, and a local inflammatory state is likely induced at this interface between the endometrium and myometrium.

Bourdon and coworkers presented a systemic review regarding the immunological changes associated with adenomyosis. They showed an imbalance between pro-inflammatory (IL1β, IL-6, IFNs, MCP1: *CCL2*, etc.) and anti-inflammatory factors (IL10, TGFβ, IL22, etc.), with the increased expression levels [12]. An altered local immune environment with a deregulated balance influenced by these factors could promote the migration of endometrial cells into the myometrium. Table 1 shows the representatives of the 84 essential genes concerning the inflammatory cytokines and receptors by DNA array. In these factors such as several interleukins, interferon, CCLs (chemokine ligand), and their receptors, IL-6 was not included. Among the upregulated genes in the endometrium with adenomyosis, we noticed CCL26 expression, a potential cytokine. Because IL1β, a possible key factor, was significantly upregulated in this study, our present data which CCL26 expression was enhanced in the endometrium with adenomyosis could be regarded as conceivable.

A previous report has assessed the gene expression levels in proliferative endometrium with adenomyosis by endometrial sampling [13]. We chose to examine the gene profiles in the secretory phase because we had predicted that abnormal decidualization and endometrial dysfunction in the secretory phase would affect the gene profile in the endometrium of adenomyosis. Another study comparing the endometrium of patients with adenomyosis and those without adenomyosis reported that the EMT process occurs in the endometrium of secretory phase with adenomyosis [14]. It is known that CCL26 is strongly expressed in

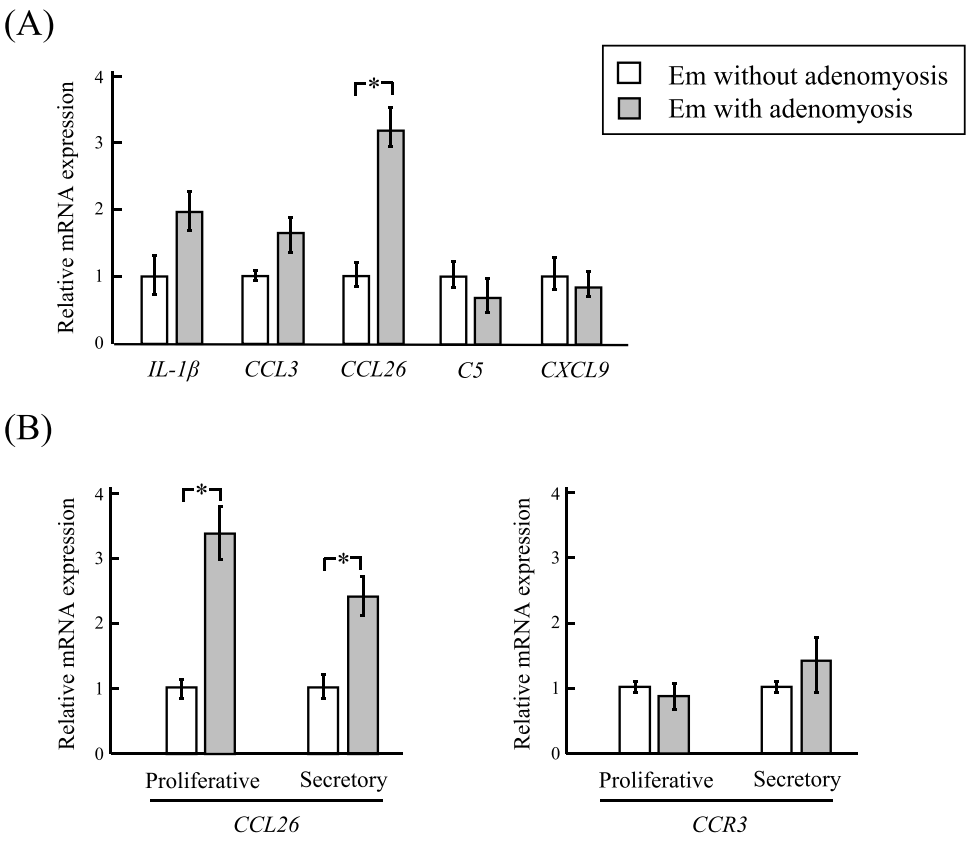

Fig. 1. Relative mRNA expression levels of inflammatory cytokines in the endometrial tissues (Em) by real-time RT-PCR.
(A) Gene expression levels of *IL-1β, CCL3, CCL26, C5,* and *CXCL9*, and (B) CCL26 and CCR3 during the menstrual phases in the endometrial tissues without adenomyosis (white: Em without adenomyosis, $n = 7$; proliferative phase, $n = 3$; secretory phase, $n = 4$) or with adenomyosis (gray: Em with adenomyosis, $n = 12$; proliferative phase, $n = 4$; secretory phase, $n = 8$). The expression levels were normalized to *GAPDH*. Results are expressed as a ratio to the values of the control, i.e., the endometrial tissues without adenomyosis. $^*p < 0.05$, a statistically significant difference between the groups. *IL-1B*: interleukin-1β, *CCL3*: chemokine ligand 3, *CCL26*: chemokine ligand 26, *C5*: complement C5, *CXCL9*: chemokine ligand 9.

Table 2
Characteristics of the patients included in the immunohistochemical analysis.

Characteristics	Adenomyosis (n = 37)		Group C Control (n = 19)
	Group A GnRHa (-) (n = 19)	Group B GnRHa (+) (n = 18)	
Age (years)	44.7 ± 4.2 (37 – 52)	44.6 ± 3.9 (38 – 53)	37.2 ± 6.7 (27 – 50)
Gravida	2.8 ± 2.4 (0 – 11)	2.5 ± 1.6 (0 – 5)	2.5 ± 2.2 (0 – 9)
Parity	1.4 ± 1.0 (0 – 4)	1.8 ± 1.3 (0 – 4)	1.3 ± 1.0 (0 – 3)
Phase (p/s/m)	9/10/0		6/12/1
NRS before surgery	6.9 ± 3.2 (0 – 10)	5.3 ± 3.3 (0 – 10)	
Type of lesions (intrinsic/extrinsic)	13/6	15/3	
Coexisting diseases (endometrioma/fibroid)	8/7	4/6	0/2

Note: Data are expressed as the mean ± standard deviation (range), or number. Abbreviations: GnRHa, gonadotropin-releasing hormone agonist; p, proliferative phase; s, secretory phase; m, menstrual phase; NRS, numeric rating scale.

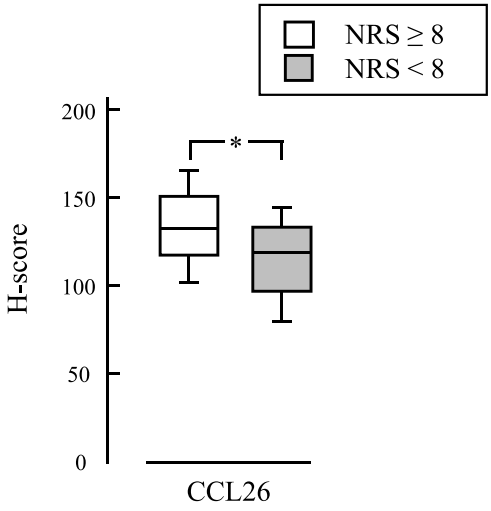

Fig. 2. Correlation between CCL26 expression and pain score.
The pain score was assessed using the numeric rating scale (NRS) and divided as follows: 8 points or more ($n = 18$) or less than 8 points ($n = 19$). Boxplot of the H-score indicates the expression of CCL26 in the group with adenomyosis. Solid vertical lines indicate the median values. Bars represent the mean value ± standard error.

human endometrial epithelial cells and that it acts predominantly as a CCR3 agonist. CCR3 has also been identified in human eosinophils throughout the menstrual cycle [15], indicating that the CCL26-CCR3 axis may be crucial for the function of the endometrium with adenomyosis.

Two prevailing theories for the pathogenesis of adenomyosis have been proposed: invagination and metaplasia [16,17]. The most widely accepted one is that adenomyosis develops as a down-growth and invagination of the basalis endometrium into the myometrium. In the invagination hypothesis, based largely on TIAR theory, TIAR in the junctional zone with adenomyosis is activated by repeated tissue microtrauma [4,18]. Chronic proliferation and inflammation induced at the level of the archimetra due to uterine auto-traumatization may be involved in the pathophysiology of adenomyosis [4]. Several investigators showed the following findings concerning the involvement of EMT in adenomyosis. Adenomyosis may be promoted by EMT of endometrial epithelial cells induced by macrophages that incapably polarize to M2 [14]. Qi et al. described the possible involvement of Notch1/Snail/Numb signaling, an EMT-related pathway, in the

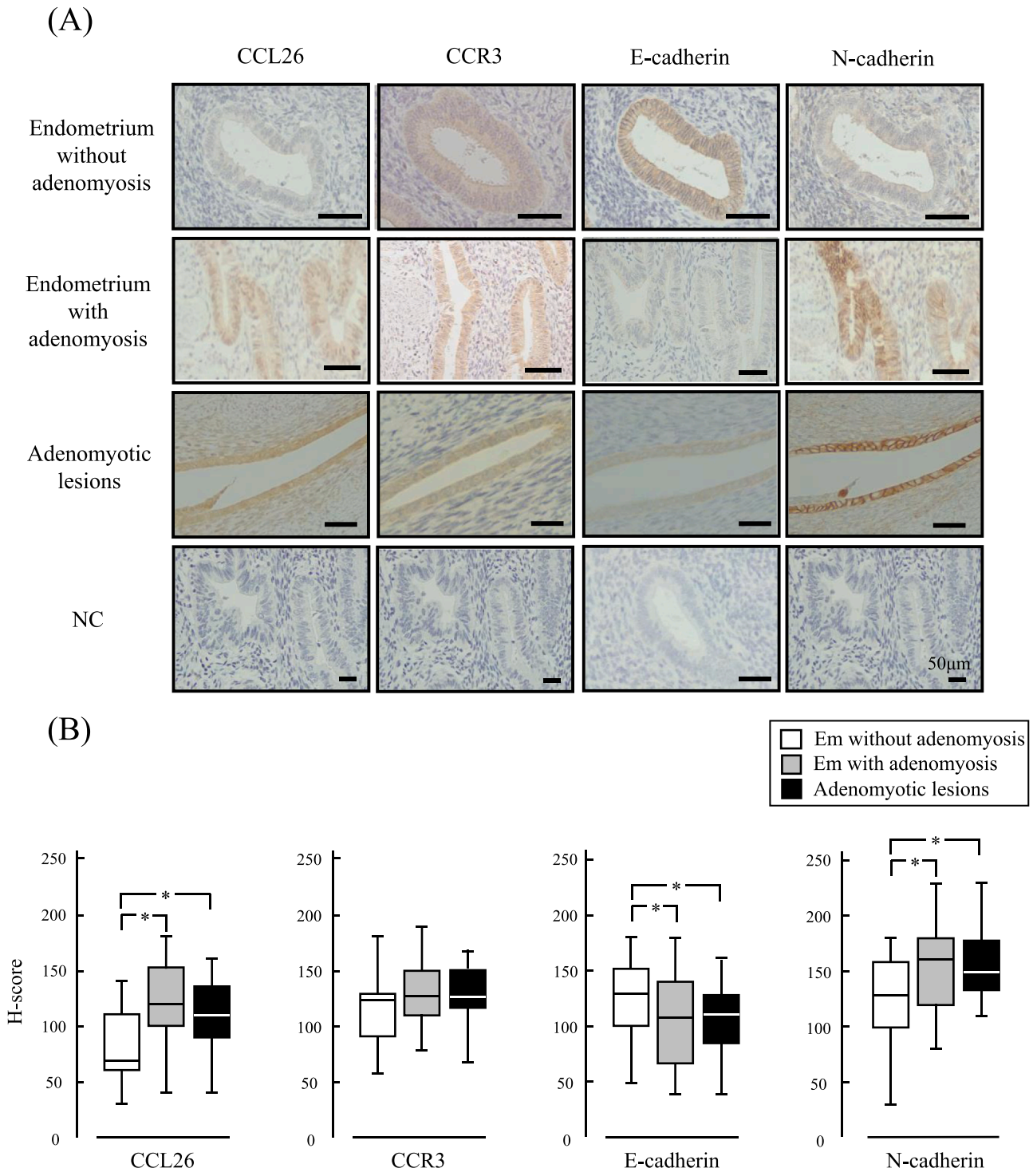

Fig. 3. Immunohistochemical analysis of CCL26, its receptor, and cadherins in the basal layer of the endometrium.
(A) Representative photos and (B) comparison of CCL26, CCR3, E-cadherin, and N-cadherin staining in the endometrium with or without adenomyosis, or in adenomyotic lesions. Horizontal bars in photos represent 50 μm. Slides with no primary antibody were used as the negative controls (NC). Boxplots of H-score (Y-axis) indicate the immunostaining levels in endometrium without adenomyosis (white; $n = 19$), with adenomyosis (gray; $n = 37$), or in adenomyotic lesions (black; $n = 19$). (C) Correlation between the expression levels of CCL26 and E-cadherin or N-cadherin. Scatterplots of H-score indicate the relation between CCL26 and E-cadherin or N-cadherin. Regression analyses with straight-line approximations are shown. R2: coefficient of determination. (D) Expression levels of these factors in the endometrium of adenomyosis patients with or without GnRHa treatment before hysterectomy. Boxplots of H-score indicate the expression levels in the endometrium without (gray; $n = 18$) or with (black; $n = 19$) GnRHa pretreatment. (E) Expression levels of CCL26, CCR3, E-cadherin, and N-cadherin in the endometrium of adenomyosis patients without ovarian endometrioma or fibroids (white; $n = 15$), with only ovarian endometrioma (gray; $n = 9$), or with only fibroids (black; $n = 10$). *$p < 0.05$, a statistically significant difference between the groups. Em: endometrium, Ad: adenomyosis, GnRHa: gonadotropin-releasing hormone agonist, H-score: Histo-score.

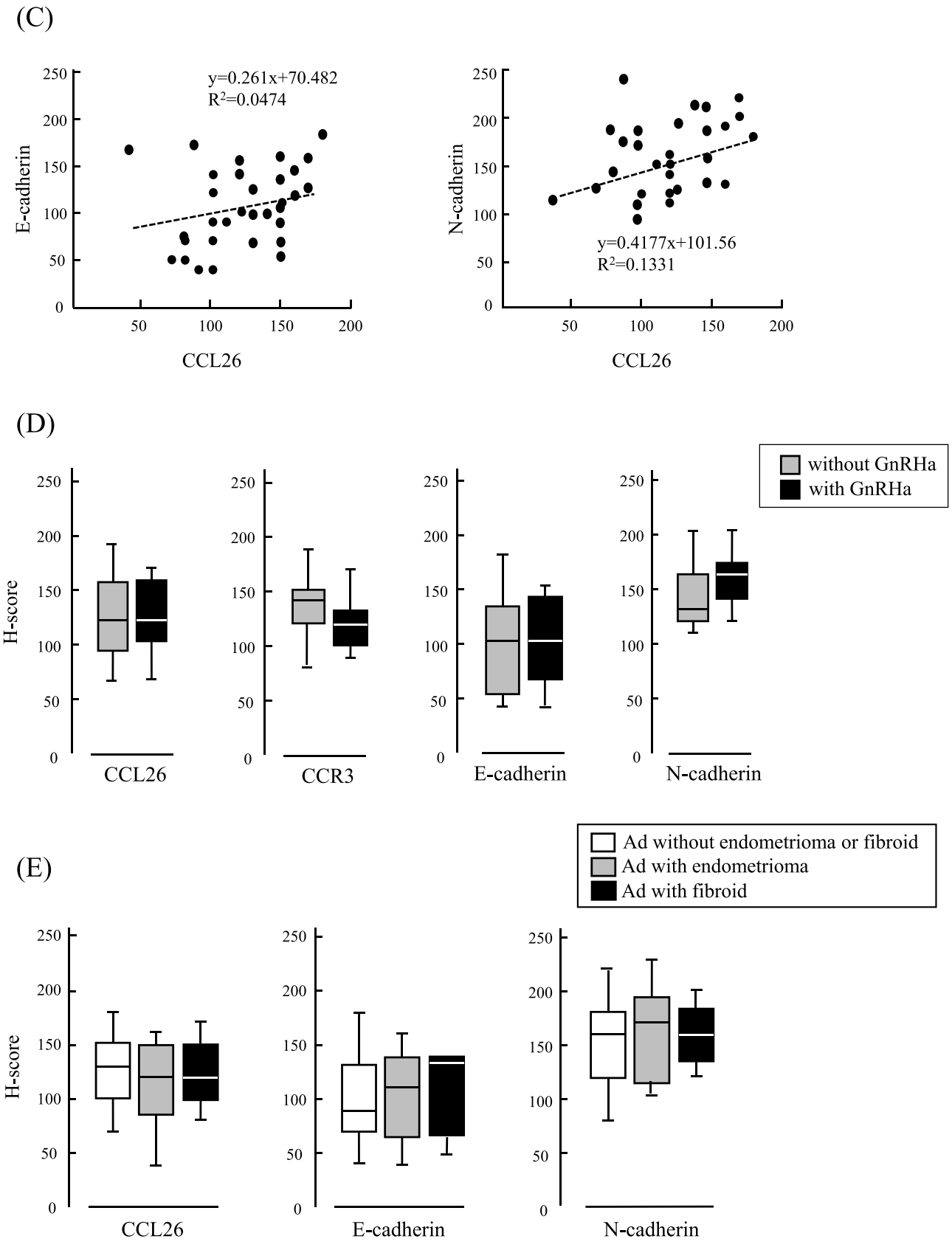

Fig. 3. (*continued*).

pathogenesis and development of adenomyosis [19]. In the murine model, Bourdon et al. presented that the activation of Notch 1 signaling pathway coincides with aberrant expression of EMT markers in the early development of adenomyosis [20]. Recently, a new hypothesis, i.e., endometrial and myometrium interface (EMI) disruption, was proposed to explain the features of adenomyosis [16]. The CCL26-CCR3 axis may

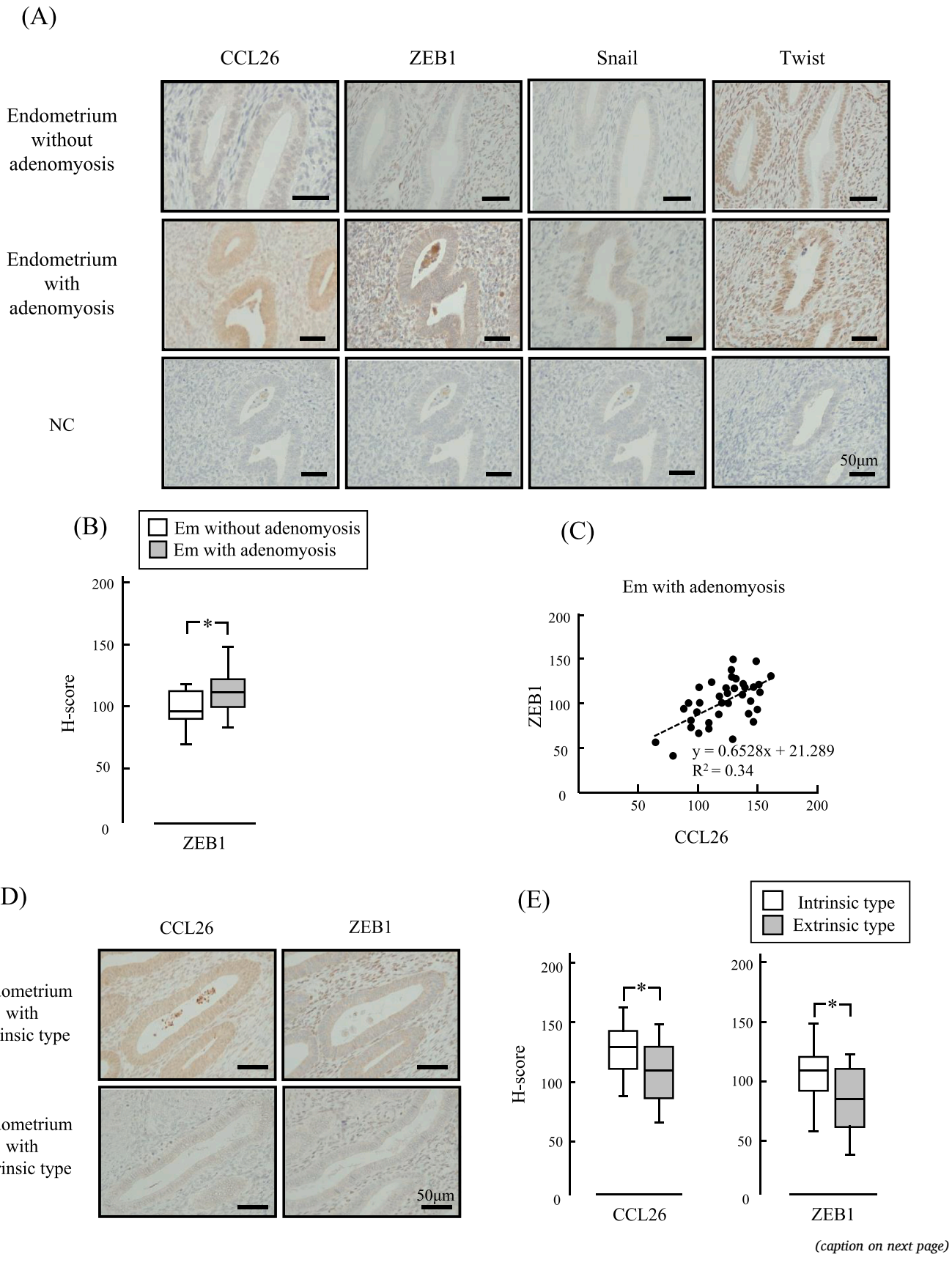

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Fig. 4. Immunohistochemical analysis of CCL26 and EMT-related transcription factors in the basal layer of the endometrium.
(A) Representative photos of CCL26, ZEB1, Snail, and Twist staining in the endometrium derived from patients with or without adenomyosis. Slides with no primary antibody were used as the negative controls (NC). Horizontal bars in photos represent 50 μm. (B) Comparison of ZEB1 expression in endometrium without or with adenomyosis. Boxplot of H-score indicates the immuno-staining levels of ZEB1 in the basal layer of the endometrium without (white; $n = 19$) or with adenomyosis (gray; $n = 37$). *$p < 0.05$, a statistically significant difference between the groups. (C) Correlation between CCL26 and ZEB1 expression in the endometrium with adenomyosis. Scatter plots of H-score indicate the relation between CCL26 and ZEB1 expression. Regression analyses with straight-line approximations are shown. R2: coefficient of determination.
(D) Representative photos and (E) comparison of CCL26 and ZEB1 staining according to the types of adenomyosis (intrinsic or extrinsic type). Horizontal bars in photos represent 50 μm. Boxplots of H-score indicate the immuno-staining levels of CCL26 and ZEB1 in the endometrium with the intrinsic type ($n = 28$) or extrinsic type ($n = 9$) of adenomyosis. *$p < 0.05$, a statistically significant difference between the groups.

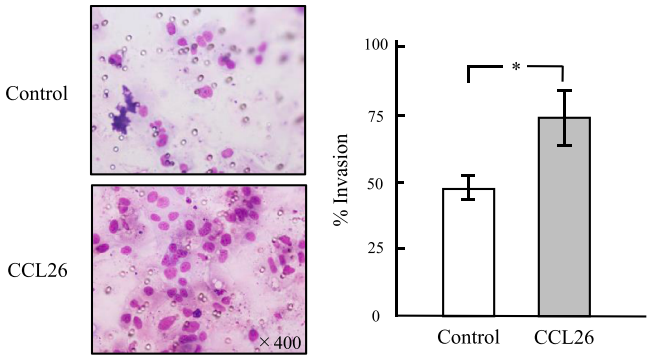

Fig. 5. The effect of exposure to CCL26 on the invasion of Ishikawa cells, an endometrial epithelial cancer cell line, was used. The invasion of cells was determined as the number of cells traversing micropores for 24 h. The rate of invasion (%) was calculated as: [the number of cells that invaded the Matrigel / the number of cells that migrated to the control insert × 100]. *$p < 0.05$, a statistically significant difference between the groups.

induce EMT, leading to the invasion of endometrial epithelial cells into the EMI.

By classifying the adenomyosis lesions into the intrinsic or extrinsic type, we revealed that CCL26 may be involved in the process of initiating ectopic intra-myometrial endometrial tissue by EMT, especially in the intrinsic type. A previous study showed that E-cadherin expression is decreased, and N-cadherin expression is increased in the epithelial cells of adenomyotic lesions when compared to the expression levels in the epithelial cells of endometrium [19]. As expected, cadherin switching (the downregulation of E-cadherin and upregulation of N-cadherin) occurred in the basal layer of the endometrium with adenomyosis. Our data suggest that the increased CCL26 levels lead to the binding of CCL26 to CCR3, and the subsequent transmission of signals, and ZEB1 is then transferred from the cytoplasm to the nucleus.

CCL26 and cadherins reciprocally play roles in the invagination of the basal layer of the endometrium into the myometrium with adenomyosis, especially in the intrinsic type adjacent to the endometrium. Other investigators have used a different classification system of adenomyosis that consists of two subtypes, i.e., the diffuse type and focal type [21]. Because a hysterectomy was performed on these patients with severe dysmenorrhea and hypermenorrhea in this study, we found no differences in preoperative symptoms regardless of the types of adenomyosis.

Increased CCL26 expression has been found in patients with eosinophilic diseases, such as asthma, atopic dermatitis, and esophagitis [22]. We supposed that eosinophils migrate from the extravascular to the basal layer of the endometrium with adenomyosis, and they may trigger the EMT. Other groups mentioned that eosinophils in the nasal or paranasal sinus and bronchial epithelial cells introduced the EMT process [23,24]. Although there have been no reports of a direct link between allergies and adenomyosis, endometriosis patients have a higher likelihood of having allergies and the coexistence of autoimmune diseases [25,26]. There is a possibility of a common pathogenic mechanism for allergy and endometriosis because the inflammatory cytokines have been closely involved [27]. On the other hand, Chen Y-J et al. showed that the serum estrogen level was decreased in adenomyotic cases that were positive for E-cadherin expression in the endometrium [28]; however, CCL26 expression in the basal layer of the endometrium was not affected by estrogen suppression (Fig. 3D).

There are several limitations to this study. First, the present study did not include experiments in ectopic lesions, and the number of samples analyzed for gene analysis and immunohistochemical staining was small. Second, the specimens of adenomyotic and endometrial tissues collected for immunohistochemical staining were from resected whole uteri of relatively high-aged (over 40) patients. It would be of interest to additionally examine endometrial specimens from women of reproductive age. Third, the invasion assay was conducted using the Ishikawa cell line instead of the endometrial epithelial cells. More research is needed to better understand the early events implicated in the initiation of adenomyosis and to develop appropriate therapeutic strategies.

Conclusion

CCL26 may be involved in gland invagination and invasion by inducing EMT in the basal layer of the endometrium. We revealed that CCL26 expression may be a useful finding for identifying primary or active lesions.

Declaration of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jogoh.2023.102645.

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